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Strong impacts of signal crayfish invasion on upland stream fish and invertebrate communities

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Abstract

1. Impacts of invasive signal crayfish *Pacifastacus leniusculus* on native species and ecosystems are widely recognised, but mostly through small-scale studies and laboratory experiments that may not always reflect impacts in nature. Recorded effects of signal crayfish on fish populations are equivocal. In this study, using the before–after/control–impact and control–impact approaches, the effects of signal crayfish invasion on native fishes, particularly benthic fishes and young-of-year (YoY) salmonids, and macroinvertebrate communities, were determined on several spatial and temporal scales through three correlated study elements (S_1–S_3), in upland streams of the River Tees, England.
2. In S_1, we sampled fish and benthic macroinvertebrates of 18 streams identically in 2011 and 2018. These streams were categorised into two groups: (1) uninvaded (without signal crayfish in both sampling years; $n = 7$); and (2) invaded (with signal crayfish) streams, comprising pre-invaded (invaded before 2011; $n = 8$) and newly invaded (invaded between 2011 and 2018, $n = 3$). Despite similar habitat conditions in both years (all variables $p > 0.05$) fish and macroinvertebrate communities changed over time in pre-invaded streams and by comparison to uninvaded streams. A decline in the abundance of benthic fish and YoY salmonids was observed in pre-invaded and newly invaded streams. Complete disappearance of bullhead *Cottus perifretum* following signal crayfish invasion was recorded in two pre-invaded streams.
3. In the second study, S_2, we assessed within-stream differences in fishes and macroinvertebrates in two Tees streams by comparing sections with (invaded) and without (uninvaded) signal crayfish. Compared to uninvaded sections, taxonomic richness and abundance of fish and macroinvertebrates were significantly lower in invaded sections, and the overall communities also differed significantly.
4. In S_3, long-term data series (since 1990) of water quality and macroinvertebrates of six Tees streams comprising those invaded by signal crayfish ($n = 3$) and uninvaded ($n = 3$) were analysed. Water quality showed little change, or an improvement, over time but significant changes in the macroinvertebrate taxonomic richness and community structure occurred following signal crayfish invasion. Long-term changes in macroinvertebrate communities in invaded streams tended to be due to declines in more sedentary taxa such as molluscs and cased trichopterans.

5. Widespread and long-term ecological disruption is occurring because of signal crayfish invasion in upland streams of the Tees catchment that may lead to a complete disappearance of some benthic fish species, as well as reduced densities of YoY salmonids and a shift towards less diverse macroinvertebrate communities, dominated by more mobile, crayfish-resistant taxa.

KEYWORDS

benthic fish, biological invasion, conservation, non-native species, salmonid

1 | INTRODUCTION

Among the reasons for declining biodiversity worldwide, biological invasion plays a key role (Dudgeon et al., 2006; Naeem, Duffy, & Zavaleta, 2012; Simberloff et al., 2013). Non-native species can impact invaded ecosystems directly (e.g. predation, competition, and displacement of native species) or indirectly (e.g. trophic cascade), resulting in altered structure and functioning of the receiving ecosystem (Bondar et al., 2005; Gutiérrez et al., 2014; Strayer, 2010).

Crayfish are a key group of invasive species and considered a major threat to native communities in freshwater habitats across the globe (Light et al., 1995; Matsuzaki et al., 2012; Usio et al., 2001). Crayfish are among the largest and longest-lived freshwater invertebrates and where they occur they often form a major part of benthic biomass (Lodge & Hill, 1994; Sousa et al., 2013). They are omnivorous and their diet may include invertebrates, macrophytes, periphyton, detritus, fish, and other crayfish (Lirås et al., 1998; Momot, 1995; Nyström et al., 1996). They are commonly considered as ecosystem engineers, partly because of their role in the alteration of detrital processing rates (Carvalho et al., 2016; Creed & Reed, 2004), and by grazing plants (Creed, 1994; Matsuzaki et al., 2009; Nyström et al., 2001) and so are also considered keystone consumers (Gheradi et al., 2011; Reynolds, 2011).

Non-native crayfish can alter aquatic biota directly, and indirectly through complex interactions (Jackson et al., 2014; Reynolds, 2011; Ruokonen et al., 2014). Their effects may be extensive if they grow to a large size or populations become dense (Gherardi et al., 2011; Strayer, 2010), resulting in an alteration of community composition and functioning (Jackson et al., 2014). Other crayfish species, macroinvertebrates, molluscs, benthic fishes, amphibians, and macrophytes are vulnerable to non-native crayfish invasion (Dorn, 2013; Gherardi et al., 2011; Mathers et al., 2016; Wilson et al., 2004). Non-native crayfish serve as vectors of parasites and pathogens such as crayfish plague *Aphanomyces astaci*, which can drastically reduce the abundance of native crayfishes (Lodge et al., 2012). Reduced growth rate and feeding of native fish species have been reported in habitats with non-native crayfish (Light, 2005) although in some cases with fish predators of invasive crayfish, predatory fish growth has increased (Wood et al., 2017).

Signal crayfish (*Pacifastacus leniusculus*, Dana) is one of the most widespread invasive crayfishes (Holdich, 2002; Lewis, 2002). It exhibits wide tolerance physiologically (Bubb et al., 2002; Lirås et al., 1998; McMahon, 2002) and ecologically (Holdich et al., 1999; Karjalainen et al., 2015), as well as a strong dispersal propensity (Bubb et al., 2006), aiding rapid colonisation. Rapid growth, early maturation, and greater fecundity also make it a successful invader (Westman & Savolainen, 2001). Although impacts of invasive crayfishes, including signal crayfish, on fishes are known, some evidence is contradictory. Small benthic fishes (cottids and loaches) can be less abundant in river reaches invaded by signal crayfish than without (Bubb et al., 2009; Guan & Wiles, 1997) and similarly for brown trout (*Salmo trutta*) in headwater streams (Peay et al., 2009), although these data were correlative and measured over short time scales. Other studies found no effect of signal crayfish on trout density (Degerman et al., 2007). Research on egg and alevin predation by signal crayfish (Edmonds et al., 2011; Findlay et al., 2015), has suggested that impacts on salmonids are likely to be most evident in the first year of life, reflecting survival from the spawning redds, since in salmonids subsequent survival is strongly density dependent, and densities may also alter due to migration (Findlay et al., 2015). However, to date, no study has measured the relationship between young-of-year (YoY) salmonids and signal crayfish in the wild. Laboratory experiments have revealed that signal crayfish can outcompete benthic fish species (sculpin, *Cottus* sp.; and stone loach, *Barbatula barbatula*) for shelter and significantly increase mortality of benthic fish (Guan & Wiles, 1997). However, small-scale laboratory experiments may not be appropriate for predicting the impacts of crayfish in nature (Degerman et al., 2007). In field studies, it is difficult to determine factors, including invasive crayfish, responsible for changes in fish populations, without controlling for habitat and year-to-year recruitment variability, and this issue has not been sufficiently addressed (Degerman et al., 2007; Peay et al., 2009).

Knowledge of the impacts of crayfish on biodiversity and ecosystem services is important for formulating management strategies (Jackson et al., 2014; Lodge et al., 2012; Moorhouse et al., 2014). Although several studies have examined the community-scale impacts of invasive crayfishes (Jackson et al., 2014; Rosewarne et al., 2016; Stenroth & Nyström, 2003) most have been short-term and utilised mesocosm experiments. Responses to invasion have mostly been analysed from spatial comparisons (with vs. without invader e.g. Crawford et al., 2006; Ercoli et al., 2015). These do not provide information on temporal invasion impacts. Mathers

et al. (2016), examining long-term impacts of signal crayfish on lotic macroinvertebrate communities, used a paired control (uninvaded)–intervention (invaded) design of study sites to minimise the likelihood of differences in water quality or stream habitat as being causal in observed changes in invertebrate communities at sites invaded by signal crayfish. However, that study considered lowland rivers and, therefore, its results cannot be extrapolated to upland streams, which differ in habitat and hydrology from lowland streams. Accordingly, there is a need for longer-term studies of the impacts of invasive crayfish on upland streams. Ideally such studies should cover multiple generations of focal species and employ before–after/control–impact (BACI) methodology, to determine the impact of invasive species such as signal crayfish. Due to the potential for reaching *tipping points* due to biodiversity loss (Dirzo et al., 2014), determining the extent of ecological impact due to species invasion should also measure the response of multiple taxa such as plants, invertebrates, and fishes.

Based on available evidence, we hypothesised that, in upland streams, non-native signal crayfish would negatively affect those community components most likely to be susceptible to benthic interactions with crayfish, these being small benthic fishes, YoY salmonids and less mobile macroinvertebrate taxa. We also hypothesised that invasion-mediated faunal impacts operate on a timescale reflective of the period taken for signal crayfish colonisation to achieve densities approaching carrying capacity. To test these hypotheses, we measured the impacts of non-native signal crayfish through three correlated studies, using BACI or control–impact study designs, with consideration of habitat and water quality factors, on native fish populations and invertebrate communities in upland U.K. streams. By doing so, we show the effects of crayfish on native communities in invaded streams, compared to uninvaded habitats, on several spatial and temporal scales.

2 | METHODS

2.1 | Study area and approach

Here, three correlated studies (hereafter S_1–S_3) were undertaken to evaluate moderate- to long-term impacts of signal crayfish within upland stream habitats. In S_1, 18 streams of the upper to middle River Tees catchment in north-east England were surveyed identically for fish and benthic macroinvertebrates in 2011 and 2018 (Table 1, Figure 1). The Tees has an upland limestone geology, with a hydrological regime dominated by rapid surface run-off in response to rainfall, and riffle-pool streams dominated by larger sediments (cobble, boulder). Historically, large parts of the Tees catchment were inhabited by native white-clawed crayfish (*Austropotamobius*

TABLE 1 Location and characteristics of streams sampled in the Tees catchment, classified by invasion condition in 2018

Site number	Stream names	Location	Area sampled (m^2)	Width (mean ± *SD*; m)		Stream categories[a]
				Channel	Wetted	
1	Parkend Beck	54°37′42″N 2°06′54″W	106.1	4.4 ± 2.1	2.9 ± 1.5	Uninvaded
2	Unnamed Beck	54°37′24″N 2°06′38″W	127	1.5 ± 0.6	1.2 ± 0.4	Uninvaded
3	River Lune	54°37′05″N 2°03′20″W	147.2	10.3 ± 0.4	9.5 ± 1.0	Newly invaded
4	Icaron Beck	54°36′26″N 2°02′07″W	107.2	3.2 ± 0.8	2.3 ± 0.6	Pre-invaded
5	Blackton Beck	54°37′02″N 02°01′00″W	119.3	3.7 ± 0.3	3.1 ± 0.5	Uninvaded
6	Wilden Beck	54°34′50″N 01°59′44″W	136.8	3.8 ± 0.8	3.3 ± 0.8	Pre-invaded
7	River Balder	54°34′31″N 01°59′13″W	97.8	11.1 ± 0.4	9.8 ± 0.5	Pre-invaded
8	Lance Beck	54°34′11″N 01°57′53″W	130.3	4.5 ± 0.7	3.4 ± 0.5	Pre-invaded
9	Scur Beck	54°33′03″N 01°56′21″W	176.8	6.1 ± 1.9	5.8 ± 2.1	Pre-invaded
10	Deepdale Beck	54°32′42″N 01°55′56″W	121.0	10.7 ± 0.9	5.2 ± 0.5	Pre-invaded
11	Thorsgill Beck	54°31′55″N 01°54′19″W	147.8	3.7 ± 0.7	2.4 ± 0.9	Newly invaded
12	River Greta	54°29′45″N 01°55′46″W	126.4	7.7 ± 0.7	7.2 ± 0.8	Uninvaded
13	Gill Beck	54°29′21″N 01°54′18″W	109.2	5.2 ± 1.1	3.7 ± 1.4	Uninvaded
14	Sudburn Beck	54°34′32″N 01°47′20″W	145.9	4.4 ± 0.5	4.1 ± 0.8	Newly invaded
15	Alwent Beck	54°33′35″N 01°46′28″W	212.2	7.2 ± 0.5	6.1 ± 1.0	Pre-invaded
16	Westholme Beck	54°33′24″N 01°46′45″W	121	2.7 ± 0.9	2.3 ± 0.6	Pre-invaded
17	Aldbrough Beck	54°30′15″N 01°41′51″W	150.7	5.4 ± 0.8	3.9 ± 1.2	Uninvaded
18	Clow Beck	54°29′21″N 01°37′21″W	166.3	6.2 ± 0.6	6.2 ± 0.6	Uninvaded

Note: The same sites were surveyed in 2011, providing a BACI sampling methodology. Site numbers refer to those in Figure 1.

[a]Based on signal crayfish invasion status. Pre-invaded sites where signal crayfish invaded before 2011, newly invaded sites, invaded by signal crayfish between 2011 and 2018, and uninvaded sites, with no signal crayfish recorded or known.

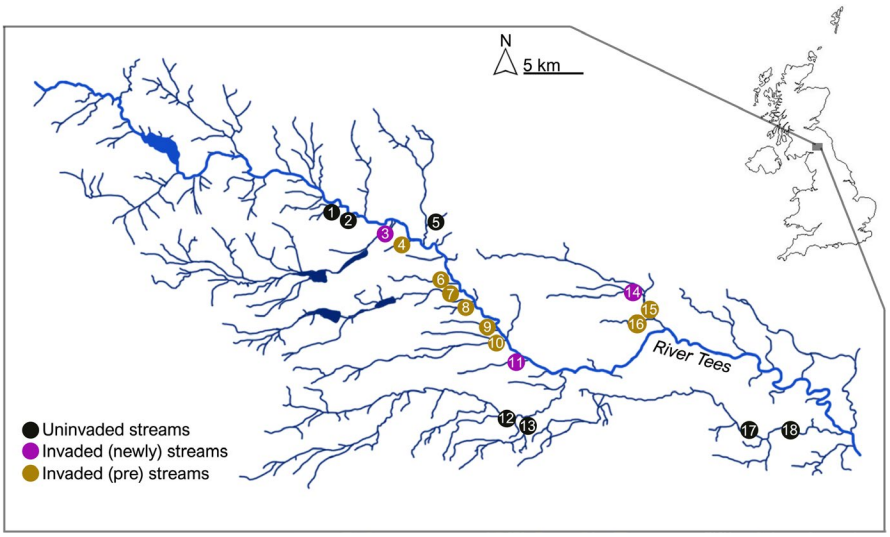

FIGURE 1 Map of the study site locations in the River Tees catchment of north-eastern England. Stream names, grid coordinates and characteristics are given in Table 1

pallipes) but several mass mortalities were recorded in the 1980s and the species had declined dramatically by the 1990s and was almost completely replaced in the 2000s by signal crayfish (Holdich et al., 1995, 1999; Priestley, 2003). White-clawed crayfish were not found at any of this study's survey sites in 2011 and 2018. Tributary streams provided environments that could be sampled quantitatively for crayfish and fish whereas the main river channel could not. Tributary streams also provided sampling units that were relatively independent from one another, since in most of those invaded by signal crayfish, it is likely that signal crayfish used the main River Tees as a conduit for stream colonisation, given the location of the original stocking site (ponds in a tributary of Deepdale Beck, Figure 1, Table 1) and the known Tees invasion history (M.C. Lucas, personal observation). The surveyed streams were divided into two groups, depending on the status of signal crayfish invasion, comprising: (1) uninvaded streams, streams with no signal crayfish over 2011–2018; and (2) invaded streams. Invaded streams were further divided into two types: pre-invaded streams—streams invaded by signal crayfish prior to 2011; and newly invaded streams—streams invaded by signal crayfish between 2011 and 2018 (Table 1).

The second study (S_2) was conducted in Thorsgill and Alwent Becks (Figure 1) in 2018, both part of study S_1. Fish and macroinvertebrate sampling were carried out at signal crayfish-invaded and uninvaded sections within the same stream. In S_3, we analysed macroinvertebrate data (1990–2018) and historical water quality (1990–2018) of six of the streams surveyed in S_1, comprising uninvaded and invaded categories. Combination of these three studies enabled robust evaluation of impacts of non-native signal crayfish on native fish and macroinvertebrate communities in upland streams over different temporal and spatial scales, through which to test our hypotheses.

We employed a BACI approach in S_1 and S_3 (Boys et al., 2012; Galib et al., 2018; Galib, et al., 2018) and a control–impact approach in S_2, where sampling years represent time *before–after* and status of signal crayfish (present or absent) in streams represents *control* (i.e. uninvaded streams) and *impact* (i.e. invaded streams) sites.

2.2 | Sampling

2.2.1 | Fish and signal crayfish sampling

In S_1, in summer 2011 and 2018, fish, signal crayfish and benthic macroinvertebrates were sampled at the same site for each stream during base-level water flows. A wetted area of between 97.8 and 212.2 m^2 comprising riffle/cascade, glide and pool habitat was surveyed at each site (Table 1). Fish densities were estimated from depletion sampling (three runs, minimum period between runs, 15 min) using electrofishing by wading. Stop nets were placed at the boundaries of the sampling reach. After each electrofishing run, fish species were identified, counted and their lengths were measured before releasing them outside of the fished area. Densities were calculated by the method of Carle and Strub (1978). In S_2, fish densities were estimated at both crayfish-invaded and uninvaded sections of Thorsgill Beck and Alwent Beck employing the method described above.

In both S_1 and S_2, signal crayfish were caught during electrofishing for fish and by subsequent refuge-searching methods using hand-nets in the same survey areas, because electrofishing only is not a sufficiently quantitative method for crayfish survey in rocky streams, even if it can be useful for determining presence versus absence (Cowx & Lamarque, 1990; Gladman et al., 2010). Manual searching of potential refuges and crayfish capture by hand-net (Bubb et al., 2005), was carried out for 1 hr (by one experienced person) or 30 min (by two experienced persons), after electrofishing, covering a full range of sediment sizes available and used by crayfish. Crayfish catches from electrofishing and refuge searching were combined in order to calculate the minimum density of crayfish at each sampling site per standard unit of effort. Although standardised in format and enabling direct comparisons of relative abundance, the sampling did not allow population estimation. Nevertheless, we demonstrated a strong positive correlation ($R^2 = 0.76$) between density estimates obtained from handnet-searching and Surber sampling (Methods S1). All crayfish capture methods over large areas are size-selective and undersample YoY crayfish (<10 mm CL). But a combination of manual searching and electrofishing can be

advantageous in measuring signal crayfish population size structure and relative abundance (Larson & Olden, 2016; Wutz & Geist, 2013), whereas, other commonly employed methods are less robust. For example, crayfish trapping tends to be strongly biased, with higher probability of catching larger male individuals and under representation of female and young crayfish (Larson & Olden, 2016; Wutz & Geist, 2013). Crayfish were identified, measured (carapace length, CL) using Vernier slide callipers, and sexed (for crayfish with CL > 10 mm).

2.2.2 | Macroinvertebrate sampling

In S_1, benthic macroinvertebrate samples were collected at fished sites in 2011 and 2018 by 3-min kick sampling covering all available habitats, and an additional 1-min, detailed hand search (Murray-Bligh, 1999). Immediately after collection, samples were preserved in 70% ethanol. In the laboratory, invertebrates were identified to family level, except Oligochaeta, Tipuloidea (including Tipulidae, Pediciidae. and Limoniidae), Rhyacophilidae (including Glossosomatidae), and Hydracarina, following standard identification literature (e.g. Pawley, 2011). In S_2, quantitative macroinvertebrate samples were collected from invaded and uninvaded sections ($n = 6$ in each section) of the streams using a 0.1-m^2 Surber sampler.

Although duplicated sampling in 2011 and 2018 (S_1), at the same 18 sites, provided BACI data with spatial replication across different invasion conditions, it gave limited temporal context. Therefore, in S_3, we analysed long-term (since 1990) macroinvertebrate sampling data (spring and autumn only, because of larger sample size in these seasons compared to others; $n = 162$ total) of six of the Tees tributaries in our S_1 dataset (Albdrough Beck, Clow Beck, River Greta [$n = 3$ uninvaded sites] and Deepdale Beck, River Balder, River Lune [$n = 3$ invaded sites], Figure 1). These data were obtained from the Environment Agency (EA), England, and employed the same standardised kick sampling methods as described above. The signal crayfish establishment periods in invaded streams at our sample sites, and at the EA sampling localities were identified as 1995–2000 for the Balder and Deepdale Beck, and 2012–2014 for the Lune (M.C. Lucas; personal observation). We employed mid-points of these years as the invasion year during analysis, i.e. mid-1997 (for Deepdale and Balder) and 2013 (for Lune). Similar taxonomic resolution in analysis of EA data was used as described above except that in EA data oligochaetes were resolved to family level and Glossosomatidae and Rhyacophilidae were separated.

2.2.3 | Habitat and water quality characteristics

In addition to fish, crayfish, and macroinvertebrate data, we also collected and analysed habitat characteristics (flow velocity and typology, water depth, water temperature, dissolved oxygen [DO], pH, substrate composition, and canopy cover) in S_1 and S_2. In S_3, historical (since 1990) water quality data (water temperature, turbidity, biochemical oxygen demand, DO, pH, ammonia, total nitrogen, total hardness, and zinc) for two uninvaded and four invaded streams were obtained from the EA to show long-term water quality status in six of our study streams. Our aim was to provide an overview of these water quality parameters, for context of the trends occurring before and after invasion situations (see Methods S1 for details).

2.3 | Data analysis

Linear mixed-effects modelling (LMM) was employed to analyse repeated measures fish density (S_1), macroinvertebrate taxonomic richness (S_2 and S_3) and abundance (S_2) data obtained from uninvaded and invaded locations, using the *lmer* function of the *lme4* package (Bates et al., 2014); *p*-values were obtained by the *lmerTest* package (Kuznetsova et al., 2016). Two invaded stream categories in S_1 (pre- and newly invaded) were analysed separately, to more fully explore the data. During LMM, sampling years (i.e. time—before and after), sampling site categories (i.e. location; uninvaded and invaded) and their interaction (time × location) were tested as fixed effects. In S_1, sampling streams and invasion status (crayfish present/absent during sampling) were considered random effects, whereas in S_2 sampling streams was treated as a random factor, as sampling was conducted in a single year. In S_3, sampling time (months nested within years) and sampling locations (sites nested within streams) were treated as random effects. Linear mixed-effects modelling was also employed to determine changes in stream habitat characteristics (i.e. bottom substrate, flow typology, depth, and shading; using percent data for bottom substrate and flow typology; Crawley, 2013). Temporal changes (2011 vs. 2018) in various groups of interest were determined by calculating effect size, Hedges's g (Hedges, 1981) using the statistical package *effsize* in R (Torchiano, 2018). Partial correlations (Kim, 2015) were used to analyse relationships between the abundance of signal crayfish and different fish species/age classes of interest (YoY, benthic, overall salmonids) for pre-invaded streams while controlling for other species in streams.

Non-metric multidimensional scaling (NMDS; Kruskal & Wish, 1978) ordination plots were generated to visualise spatial and temporal variation of fish and macroinvertebrate community composition using the *metaMDS* function of the *vegan* package (Oksanen et al., 2018). To determine the dissimilarities among fish and macroinvertebrate communities a permutational multivariate analysis of variance (PERMANOVA), using distance matrices, was carried out by employing the *adonis2* function of the *vegan* package (Oksanen et al., 2018). Similarity percentage (SIMPER) analysis, decomposition of Bray–Curtis dissimilarity index (Clarke, 1993), was used to determine the average percent dissimilarity over time (2011 [before] vs. 2018 [after]) and to identify the contribution of individual fish species, belonging to each stream category, responsible for average dissimilarity between *before* and *after* communities in S_1. Species that accounted for the differences between *before* and *after* communities were identified from SIMPER analyses based on the ratio between the average contribution to dissimilarity and the *SD*, which is a measure of the how consistently a species contributes to dissimilarity over time (Solomon et al., 2016). SIMPER was also employed to analyse macroinvertebrate data in all three studies.

In S_1, as both fish and environmental data were available, the multivariate BIOENV procedure, based on Euclidean distances (Clarke & Ainsworth, 1993), was employed to find out the best subset of environmental variables with maximum (rank) correlation (Pearson's) with community dissimilarities (e.g. Boys et al., 2012; Galib, et al., 2018). Along with all the environmental variables (depth, flow velocity, flow typology, DO, temperature, pH, substrate, canopy cover), we also considered density of signal crayfish in the BIOENV model to determine the role of signal crayfish for changes in fish community over time.

In S_3, for three uninvaded streams (Aldbrough, Clow, and Greta), macroinvertebrate samples collected until mid-1997 were considered *before* and samples collected after 1997 were considered *after* situations. For Deepdale and Balder (invaded streams), macroinvertebrate samples before 1997 were *before* invasion, and for the Lune (invaded), samples before 2013 were *before* invasion. Four families were pooled because of variations in some aspects of taxonomic resolution through time (Limoniidae and Pediciidae were grouped under Tipuloidea; Lumbricilidae and Lumbricidae were pooled as Oligochaeta; after Durance & Ormerod, 2009). Nematoda and Hydracarina were recorded at that taxonomic resolution. As actual abundance data of macroinvertebrates were recorded on a ranked scale of logarithmic abundance, they were transformed on an ordinal scale (1 = 1–9 individuals, 2 = 10–99, 3 = 100–999, and 4 = 1,000–9,999) before analysis (after Durance & Ormerod, 2009) and used in PERMANOVA, NMDS, and SIMPER. Due to variations in macroinvertebrate sampling across studies, analyses were based on presence or absence data using biological monitoring working party scoring families (see Armitage et al., 1983 for list) in S_1, whereas abundance and categorical data were used for S_2 and S_3, respectively.

Before–after changes in historical water quality parameters were determined separately for each stream category (in S_1) and stream (in S_3) to better understand the changes in individual category or stream using LLMs with sampling stream (S_1) and months (S_3, nested within year) as random effects.

All statistical tests were carried out in R (version 3.4.3; R Core Team, 2017), with an α level of significance of 0.05. We explored data during analyses to avoid common statistical problems (Zuur et al., 2010). Before analysis data were checked for normality by Shapiro–Wilk test (Peat & Barton, 2005) and necessary transformations, square-root transformation for abundance data (McDonald, 2014) and log (x + 1) transformation for water quality data (Clarke, 1993), were made to meet the statistical assumption for the tests.

3 | RESULTS

3.1 | Fish responses to signal crayfish between and within streams

In S_1, over a 7-year period, there were no significant changes in fish abundance (all species combined) between uninvaded and newly invaded streams. Comparison between uninvaded and pre-invaded streams revealed significant time and invasion status effects on the fish community and abundance, respectively (Table 2). Pairwise comparisons showed that mean fish abundance declined by 29% in pre-invaded streams between 2011 and 2018 (p = 0.027; Table 3) but mean crayfish density increased by 93% during this time (p = 0.019, Figure 2), over the same period that abundance of YoY salmonids (p = 0.038) and small benthic fish (bullhead and stone loach, p = 0.022, Table 3) decreased by 32% and 83%, respectively (Figure 2). However, abundance of non-YoY salmonids increased by > 100% in both pre- and newly invaded streams. The overall mean minimum (±*SD*) density of signal crayfish in pre-invaded streams was 46.4 ± 31.5 crayfish/100 m^2 in 2011 and 89.7 ± 50.4 crayfish/100 m^2 in 2018. The mean minimum density in newly invaded streams was 31.4 ± 22.5 crayfish/100 m^2. Significant negative partial correlations (while controlling for other species) occurred between the abundances of signal crayfish and benthic fish (r = −0.58, p = 0.046), YoY salmonids (r = −0.78, p = 0.006), and all salmonids (r = −0.71, p = 0.010) in pre-invaded streams.

Across all streams sampled, a total of 10 fish species occurred, but brown trout and bullhead (*Cottus perifretum*, sensu Freyhof et al., 2005; formerly known as *Cottus gobio* in Britain) were the most frequently occurring fish species, the former in 17 of 18 streams and the latter in 13 streams. Changes in abundance between 2011 and 2018 were only significant for bullhead (p = 0.019) and brown trout (p = 0.024), and only in pre-invaded streams (Table 4). Bullhead contributed most strongly to differences in fish community dissimilarity between 2011 and 2018 (Table 4). Divergence in fish community composition following crayfish invasion is evident from NMDS ordination plot (Figure 3). There was no significant difference in fish communities between pre-invaded and uninvaded streams in 2011 (PERMANOVA, p = 0.11) but they differed significantly in 2018 (p = 0.002; Table S1). Fish community differed significantly in pre-invaded streams between 2011 and 2018 (p = 0.048) but did not in uninvaded streams (Table S1). No bullhead were found in two pre-invaded streams (Lance Beck and Westholme Beck) in 2018 where they were abundant in 2011. Pairwise effect size analyses also confirmed a negligible to small temporal effect size in uninvaded streams, but a small to large effect size in newly and pre- invaded streams (Table S2).

In uninvaded streams there was no difference in size (indicative of age) structure of bullhead between 2011 and 2018, with good recruitment of younger age groups into the population in both years (Figure 4). By contrast, in pre-invaded streams there was a highly significant difference (Mann–Whitney U test: U = 4,889.5, p < 0.001) between 2011 and 2018, with negligible numbers of young and evidence of recruitment failure over several years up to and including 2018, during which time crayfish increased in abundance. The same analysis for brown trout (Figure 4) showed no significant difference in size structure of trout between 2011 and 2018.

Fish abundance differed between uninvaded and invaded sections of two streams in 2018 (S_2; Thorsgill and Alwent becks; F = 254,

TABLE 2 Before–after (BA) control–impact (CI) comparison of fish and macroinvertebrate communities over time and space, obtained through permutational multivariate analysis of variance (for community) and linear mixed-effects modelling (for abundance and richness)

			Community		Abundance		Richness	
Studies	**Groups**	**Comparisons**	***F***	***p***	***F***	***p***	***F***	***p***
Study I (S_1) (uninvaded vs. newly invaded)	Fish	Time (BA)	0.5	0.671	0.5	0.517	1.0	0.342
		Invasion status (CI)	0.5	0.652	0.5	0.474	1.2	0.300
		Interaction (BA × CI)	0.6	0.681	0.5	0.503	0.1	0.865
	Macroinvertebrate	Time (BA)	1.6	0.140	–	–	–	–
		Invasion status (CI)	2.6	**0.015**	–	–	–	–
		Interaction (BA × CI)	0.7	0.730	–	–	–	–
Study I (S_1) (uninvaded vs. pre-invaded)	Fish	Time (BA)	0.9	**0.043**	0.8	0.384	1.4	0.265
		Invasion status (CI)	2.3	0.176	10.8	**0.006**	0.2	0.712
		Interaction (BA × CI)	0.1	0.774	1.0	0.343	0.01	0.965
	Macroinvertebrate	Time (BA)	5.7	**0.005**	–	–	–	–
		Invasion status (CI)	3.5	**0.010**	–	–	–	–
		Interaction (BA × CI)	0.9	**<0.001**	–	–	–	–
Study II (S_2)	Fish	Invasion status (CI)	0.6	0.500	254	**0.039**	1.0	0.500
	Macroinvertebrate	Invasion status (CI)	5.9	**0.010**	33.6	**<0.001**	17.0	**<0.001**
Study III (S_3)	Macroinvertebrate	Time (BA)	6.6	**<0.001**	–	–	0.6	0.457
		Invasion status (CI)	18.3	**<0.001**	–	–	4.6	**0.050**
		Interaction (BA × CI)	4.2	**<0.001**	–	–	8.4	**0.004**

Bold values indicate outcomes significant at $p \leq 0.05$.

TABLE 3 Statistical comparisons of abundance of fishes and signal crayfish over time (2011 vs. 2018) in relation to stream-invasion status by signal crayfish, obtained through linear mixed-effects modelling

Groups	**Occurrence (*n*)**	***F***	***p***	**Mean changes in abundance (%)**
Pre-invaded streams ($n = 8$)				
Overall fishes	8	7.71	**0.027**	↓ 29
Signal crayfish	8	9.30	**0.019**	↑ 93.1
YoY salmonids	4	12.59	**0.038**	↓ 31.7
Benthic fishes	7	6.95	**0.022**	↓ 83.2
Uninvaded streams ($n = 7$)				
Overall fishes	7	0.09	0.776	↑ 21.1
YoY salmonids	7	0.28	0.616	↑ 4.4
Benthic fishes	5	0.01	0.941	↑ 29.7
Newly invaded streams ($n = 3$)				
Overall fishes	3	5.65	0.141	↓ 54.3
YoY salmonids	2	1.64	0.399	↓ 61.5
Benthic fishes	3	5.87	0.136	↓ 61.3

Bold values indicate outcomes significant at $p \leq 0.05$.

Study S_1. *Benthic fishes* combines stone loach (*Barbatula barbatula*) and bullhead (*Cottus perifretum*), while *YoY salmonids* combines young-of-the-year of *Salmo trutta* and *Salmo salar*.

$p = 0.039$). Higher fish abundance (by >110%) occurred in uninvaded upstream sites compared to sites invaded by signal crayfish.

In pre-invaded streams, the proportion of crayfish categorised as large (≥35 mm CL) increased from 18.4% (mean [±*SD*] density and range: 8.2 ± 5.9/100 m^2, 1.9–18.6/100 m^2) in 2011 to 24.1% in 2018 (mean [±*SD*] density and range: 19.8 ± 25.1/100 m^2, 2.25–72.9/100 m^2; Figure S1). By contrast, the proportion of large crayfish was 26.4% in newly invaded streams (mean [±*SD*] density and range: 8.3 ± 6.1/100 m^2, 4–15.3/100 m^2; Figure S1). Sex ratio (male: female) of signal crayfish in pre-invaded streams was 1:1.12 and 1:1.27 in 2011 and 2018, respectively, and was 1:1.15 in newly invaded streams (2018), based on a total of 1,053 sexed crayfish.

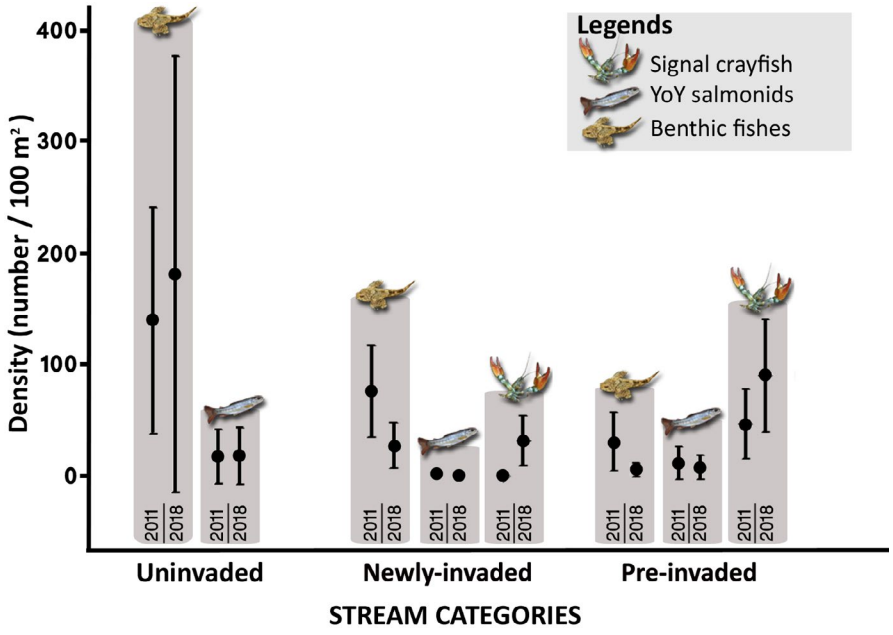

FIGURE 2 Changes in density (individuals 100 m^{-2}) of bullhead, YoY (young-of-year) salmonids and signal crayfish, (mean ± *SD*) between 2011 and 2018 across sites at different invasion stages. Fish densities measured by depletion sampling; crayfish are minimum densities per standardised effort (see text for more detail). *Newly invaded* refers to sites where signal crayfish were absent in 2011 but present in 2018; *pre-invaded* refers to sites where signal crayfish were present in 2011 and 2018

3.2 | Macroinvertebrates

Negative impacts of signal crayfish on macroinvertebrates were recorded between uninvaded and invaded locations within the same stream (S_2) or between streams over moderate to long timescales (S_1 and S_3). Within the same stream, macroinvertebrate community, taxonomic richness and abundance differed significantly between crayfish-invaded and uninvaded downstream sections (S_2; all $p \leq 0.01$; Table 2, Figure 3). Higher invertebrate abundance (by >125%) was recorded in uninvaded upstream sites compared to invaded sites. High invertebrate taxonomic richness was also recorded in uninvaded upstream sites (mean ± *SD*, 15.1 ± 2.4 families) compared to invaded downstream sites (11.1 ± 2.5 families).

Significant effects of time, invasion status and interaction were found (PERMANOVA; all $p \leq 0.01$) when comparing uninvaded and pre-invaded streams in S_1. By contrast, only a significant effect of invasion status ($p = 0.015$) was evident between uninvaded and newly invaded streams (Table 2). Pairwise comparisons showed that the invertebrate communities recorded in both 2011 and 2018 differed significantly between uninvaded and pre-invaded streams (PERMANOVA, 2011: $F = 2.77$, $p = 0.013$; 2018: $F = 3.87$, $p < 0.001$; Table S1), whereas, despite similarity in communities between uninvaded and newly invaded streams in 2011, they differed significantly in 2018 (Table S1). The community differed significantly between years in pre-invaded streams ($p < 0.001$) whereas it did not in uninvaded and newly invaded streams (Table S1) reflecting an ongoing trajectory of separation in community characteristics between invaded and uninvaded streams (Figure 3).

When comparing long-term macroinvertebrate data (S_3), strong effects of time, location and their interaction were found for macroinvertebrate community (all $p < 0.001$; Table 2). The invertebrate community in invaded streams, compared to uninvaded streams, deviated more from its initial pre-invasion condition (NMDS, Figure 3). Pairwise comparisons showed that the macroinvertebrate communities of invaded and uninvaded streams did not differ before signal crayfish invasion (PERMANOVA: $p = 0.070$), but differed significantly after invasion ($p < 0.001$; Table S1). However, communities in both invaded and uninvaded streams changed significantly from before to after invasion (both $p < 0.05$; Table S1). For taxonomic richness, significant invasion status and interaction effects were recorded (Table 2). Pairwise comparisons revealed that, similar to community responses, macroinvertebrate taxonomic richness between invaded and uninvaded streams did not differ before signal crayfish colonisation, but differed significantly after invasion ($p = 0.041$, Table S1). Macroinvertebrates in invaded streams also differed significantly in taxonomic richness between the pre- and post-invasion period ($p = 0.047$) whereas invertebrates in uninvaded streams did not, over the equivalent periods (Table S1).

Similarity percentage analyses (studies S_1–S_3), revealed that the abundance of slow-moving taxa including Ancylidae, Sphaeridae, and Oligochaeta tended to decrease over time in invaded streams/sections whereas they tended to remain stable or increase in uninvaded streams/sections (Table 5, Tables S3, S5–S8). However, no significant changes in any invertebrate taxa over time (2011 vs. 2018) were recorded in newly invaded streams (S_1; Table S4).

3.3 | Habitat and physico-chemistry of fish survey sites over time

The habitat characteristics or water quality data collected across all three study elements (S_1–S_3) were almost universally unchanged or improved over time in terms of suitability for fishes and invertebrates adapted to well-aerated upland stream conditions (Tables 6–7; also see Figures S2–S10, Table S9 for details). In S_1, BIOENV analysis revealed that the density of signal crayfish, and proportions

TABLE 4 Before–after (2011 vs. 2018, study S_1) comparison of the abundance of fish species within crayfish stream-invasion categories and contribution of species to before–after community dissimilarity

Species	Before versus after			Average percent change in abundance	Average dissimilarity/*SD*	Average dissimilarity	Cumulative percent contribution to dissimilarity
	n	*F*	*p*				
Pre-invaded streams (*n* = 8)							
Cottus perifretum	5	8.56	**0.019**	↓ 74.9	1.24	0.20	31
Salmo trutta	7	8.99	**0.024**	↑ 64.5	1.12	0.18	58
Phoxinus phoxinus	6	0.01	0.938	↑ 62.6	1.05	0.09	72
Gasterosteus aculeatus	2	0.08	0.820	↑ 60.6	0.58	0.09	86
Barbatula barbatula	3	2.30	0.204	↓ 33.9	0.77	0.08	98
Thymallus thymallus	1	–	–	↓ 100	0.36	0.01	100
Uninvaded streams (*n* = 7)							
Cottus perifretum	5	0.01	0.944	↑ 111.4	1.34	0.28	52
Salmo trutta	7	0.03	0.860	↑ 49.2	1.42	0.11	72
Gasterosteus aculeatus	2	0.63	0.511	↑ 135.2	0.66	0.04	80
Salmo salar	2	0.07	0.819	↔	0.55	0.04	86
Phoxinus phoxinus	2	2.74	0.346	↑ 63.5	0.70	0.03	92
Barbatula barbatula	1	–	–	↑ 27.5	0.54	0.02	96
Lampetra planeri	2	1.00	0.498	↑ 2.8	0.81	0.01	98
Anguilla anguilla	1	–	–	↑ 100	0.40	0.00	99
Thymallus thymallus	1	–	–	↓ 100	0.40	0.00	100
Rutilus rutilus	1	–	–	↓ 100	0.40	0.00	100
Newly invaded streams (*n* = 3)							
Cottus perifretum	3	6.19	0.131	↓ 60.3	1.89	0.1438	32.34
Phoxinus phoxinus	2	18.46	0.145	↑ 40.7	1.22	0.0778	49.85
Lampetra planeri	1	–	–	↓ 96.8	0.79	0.0644	64.33
Barbatula barbatula	2	3.78	0.287	↓ 100	1.11	0.048	75.13
Salmo trutta	3	6.05	0.133	↑ 190.3	1.28	0.0439	85.01
Gasterosteus aculeatus	1	–	–	↓ 42.7	0.90	0.0421	94.47
Salmo salar	1	–	–	↑ 80	0.83	0.0246	100

Note: The outputs of analyses of fish species abundance have been obtained through linear mixed-effects modelling (LMM) and those for community dissimilarity obtained by similarity percentage analysis (SIMPER). *n* refers to the number of streams in which each taxon was observed. *Cottus perifretum* is part of the *Cottus* species complex and previously known in Britain as *Cottus gobio*.

Bold values indicate outcomes significant at $p \leq 0.05$

of cascade and glide habitat collectively played a key role in shaping the fish assemblage patterns (Pearson correlation, $\rho = 0.42$). Among the streams in S_3, turbidity (which would increase with elevated concentrations of suspended solids) decreased markedly in one invaded stream but increased slightly in another stream for which information was available. Nitrogen and ammonia decreased significantly across the streams (Table S9).

4 | DISCUSSION

Our hypothesis, that non-native signal crayfish would impact sensitive elements of upland stream fish and benthic invertebrate fauna, was supported by the results of this study. So too was the hypothesis that such effects develop over the extended timescale that it takes for invasive crayfish populations to become fully established. Our study provides evidence that native fish abundance and the community structure of fishes and benthic invertebrates are being strongly impacted by signal crayfish in streams of a typical English upland limestone river system. In particular, small benthic fishes such as bullhead declined in streams where signal crayfish became abundant, but those in uninvaded streams did not, while habitat remained similar between stream types and sample years.

4.1 | Population and invasion of signal crayfish in upland streams

The impacts of invaders may take time to become apparent in habitats, depending on the mode of action and whether impacts

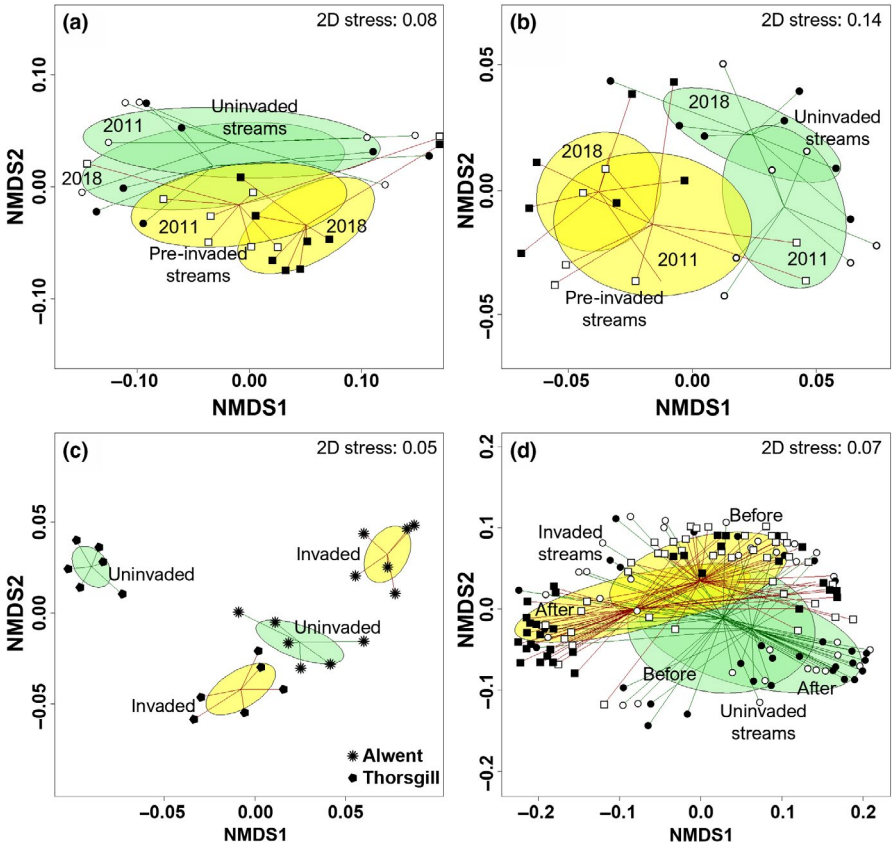

FIGURE 3 Non-metric multidimensional scaling (NMDS) ordination plots showing spatial and temporal variation of fish and invertebrate communities in invaded and uninvaded streams. (a) Fish communities between uninvaded and pre-invaded streams (study S_1). (b) Macroinvertebrate communities between uninvaded and pre-invaded streams, based on presence–absence data (study S_1). (c) Invertebrate communities between signal crayfish-invaded and uninvaded parts within in Alwent and Thorsgill becks in 2018, based on abundance data (study S_2). (d) Invertebrate communities before and after signal crayfish invasion, in three invaded and three uninvaded streams over the period 1990–2017 (study S_3). Light green and yellow ellipses are uninvaded and invaded streams/parts, respectively; open and filled symbols represent before (2011 in a and b) and after (2018 in a and b) data points, respectively (except for c). Each ellipse represents 95% confidence interval

are density-related (Simberloff et al., 2013). Few studies have presented historical timelines of changes in density of invasive crayfish following initial colonisation but, in this study, it is evident that the density of signal crayfish increased in pre-invaded streams between 2011 and 2018. Tees tributaries are being invaded quite quickly, with three sites having no signal crayfish recorded in 2011 but present in 2018 (Lune, Sudburn, and Thorsgill). Our study indicates that signal crayfish in the upper Tees are still in a population expansion phase; community impacts can therefore be expected to continue with time and are likely to become permanent.

Both size distribution and sex ratio can influence signal crayfish invasion (Light, 2003; Wutz & Geist, 2013). A higher proportion of larger signal crayfish can strongly affect stream communities and habitats, by increasing predation on fish (Guan & Wiles, 1997), and altering habitats through burrowing behaviour (Guan, 1994). Large signal crayfish are also capable of rapid, active upstream movements, facilitating colonisation (Bubb et al., 2006; Wutz & Geist, 2013). Deviation in the ratio of males to females in pre-invaded streams from the expected value of 1:1 suggests that the invasion by signal crayfish is not yet complete (Capurro et al., 2007).

4.2 | Impact on benthic fishes and YoY salmonids

BIOENV analysis, based on 2011 versus 2018 data revealed that abundance of signal crayfish was a key factor in shaping the fish communities in the invaded streams. Habitat factors were also important, but signal crayfish abundance was included in all models explaining the highest levels of variation.

Small benthic fishes such as bullhead, were particularly vulnerable to signal crayfish invasion in the Tees, with strong evidence of recruitment failure in pre-invaded streams in 2018, but no such effect in uninvaded streams. Signal crayfish can exclude benthic fishes from shelters and make them susceptible to predation (Guan & Wiles, 1997; Rahel & Stein, 1988). Bullhead seek shelter in refuges, such as under stones, during daylight (Mills & Mann, 1983), as do signal crayfish (Bubb et al., 2009). Although large sculpins can eat or displace the smallest crayfish, in European streams the competitive interaction is heavily asymmetrical in favour of signal crayfish (Bubb et al., 2009). Disappearance of bullhead in two pre-invaded streams in this study could be associated with the high density of signal crayfish, as a great reduction in benthic

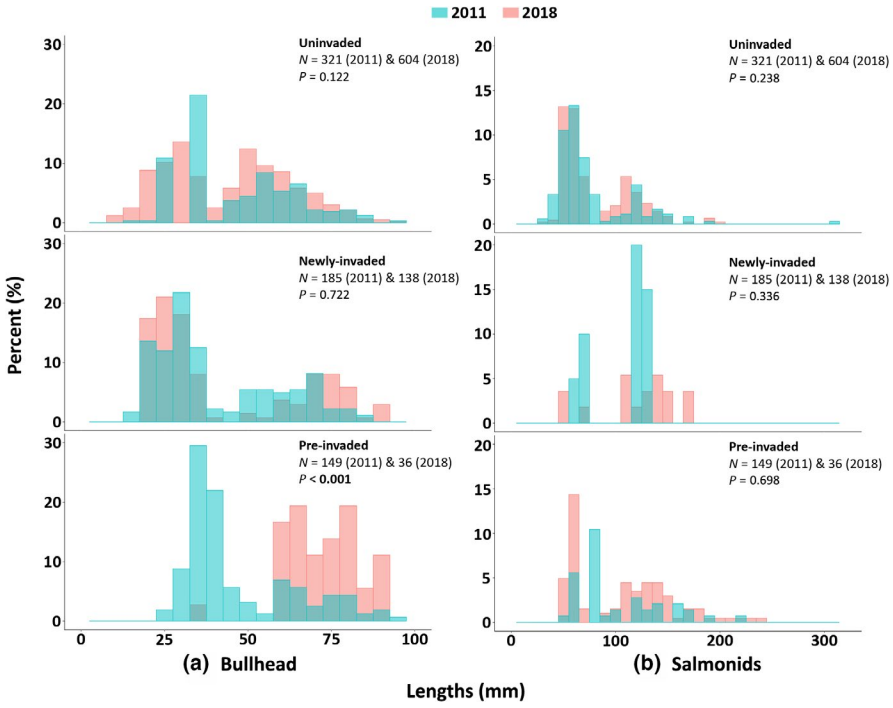

FIGURE 4 Length–frequency distributions, indicative of age distributions, of bullhead and salmonids recorded in two sampling years (2011 and 2018; S_1) in different stream-invasion categories. Note the different scales on the y-axis for each fish species. Statistical outcomes refer to Mann–Whitney *U* tests

fishes or even local extinctions are possible in habitats with a high density of signal crayfish (Bubb et al., 2009; Guan & Wiles, 1997). The population decline in sculpins is likely to be partly due to increased egg mortality since males normally guard the eggs, but may be driven away by signal crayfish (M. Lucas, personal observation), and partly to increased predation risk to juveniles and adults leaving shelter, supported by our recruitment data. These comments regarding benthic fishes are reserved for small individuals and species; larger benthic fishes such as European eel *Anguilla anguilla* can predate signal crayfish (Blake & Hart, 1995) and are unlikely to be displaced by crayfish, but eel were very rare at our study sites and occur only in low densities in the upper Tees, partly due to migration barriers further downstream.

The cause of reduced densities of YoY salmonids in crayfish-invaded streams was less clear-cut, since there was no evidence of recruitment failure. Several signal crayfish mediated factors are plausible. Firstly, salmonid eggs and alevins may be predated by crayfish (Edmonds et al., 2011; Findlay et al., 2015), although Gladman et al. (2012) found no evidence of signal crayfish detecting and digging out buried eggs from artificial redds. Secondly, crayfish compete with salmonid fry for shelter (Griffiths et al., 2004). Thirdly, an increase in fine sediment infiltration into spawning habitats due to zoogeomorphic processes involving crayfish (Harvey et al., 2011; Nyström et al., 1996) might reduce survival of salmonid eggs and alevins, which are sensitive to fine sediment (Harvey et al., 2011). However, severe negative impacts on macroinvertebrates in invaded habitats, revealed through S_1–S_3 of this study, may also have played a role in the decrease in YoY salmonids. In addition to these causes, salmonid recruitment is impacted by poor water quality, poor habitat, migration barriers, and excessive fine sediment due to poor land management (Peay et al., 2009). Interestingly, an increased number of non-YoY salmonids was recorded in invaded streams, possibly through immigration from uninvaded stream sections upstream, which suggests that crayfish invasion may be beneficial for larger individuals to some extent. A similar outcome was also reported for large chub (*Squalius cephalus*; Wood et al., 2017).

At the sites in study S_1, habitat remained relatively unchanged between 2011 and 2018. Physical obstacles are few between the main channel and the stream study sites in the upper catchment. Most water quality variables remained stable in invaded streams and so observed changes in fish and invertebrate communities are unlikely to have been driven by these. This contextual information has been missing from several past studies (Crawford et al., 2006; Mathers et al., 2016; Peay et al., 2009).

4.3 | Impact on macroinvertebrate communities

As hypothesised, strong impacts were recorded on macroinvertebrates in signal crayfish-invaded streams, with increasing effects occurring over time. Given that most temperate zone freshwater macroinvertebrates (other than crayfish and large bivalves) have a generation time of 1–3 years, and that by 2011 of study S_1, signal crayfish had probably already been in most of the pre-invaded streams for about a decade, this could already have generated a change in the community that differed from uninvaded streams in 2011. Mathers et al. (2016) showed that benthic macroinvertebrate communities typically took 5–10 years for major change to be evident following signal crayfish invasion. We did not find any time effect (2011 vs. 2018) on the invertebrate community in newly invaded streams, invaded by signal crayfish for < 7 years. However, negative impacts on taxonomic richness may take more time to become evident as no macroinvertebrate family changed

TABLE 5 Changes in abundance of the top 10 macroinvertebrate families (first 10 rows) contributing to the dissimilarity in communities before and after signal crayfish invasion (1990–2017 data, study S_3) along with several other families of concern (by reference to crayfish impact literature) in three invaded streams (Deepdale Beck, River Balder, and River Lune) and three uninvaded streams (Aldbrough Beck, Clow Beck, and River Greta) over the same period, obtained through similarity percentage analysis (also see Tables S7 and S8 for complete lists)

	Changes in abundance (%) from before to after invasion		Contribution to dissimilarity (%) from before to after invasion	
Macroinvertebrate families	Invaded streams	Uninvaded streams	Invaded streams	Uninvaded streams
Lepidostomatidae	↓ 55***	↑ 59***	3.3	2.6
Caenidae	↓ 47***	↑ 2	3.2	2.4
Ancylidae	↓ 58***	↑ 31	3.2	2.4
Perlidae	↓ 64***	↓ 41	3.1	2.0
Chloroperlidae	↑ 8	↓ 30	2.9	1.9
Sericostomatidae	↓ 15	↓ 3	2.8	2.0
Polycentropodidae	↓ 39***	↓ 46***	2.7	2.2
Limnephilidae	↓ 25***	↑ 23	2.7	2.4
Gammaridae	↑ 2	↑ 15	2.7	2.0
Leptophlebiidae	↓ 27***	↑ 53***	2.7	2.5
Sphaeriidae	↓ 38***	↑ 26	2.5	2.4
Hydrophilidae	↑ 91***	↓ 81***	2.3	1.3
Oligochaeta	↓ 19***	↑ 7	2.1	1.6
Hydrobiidae	↓ 82***	↑ 2	2.1	2.6
Gyrinidae	↓ 66***	↑ 11	2.2	2.0
Rhyacophilidae	↓18***	↓ 16	1.9	1.6
Ephemerellidae	↑ 285***	NA	1.8	NA
Glossosomatidae	↑ 306***	↑ 798***	1.7	3.7
Heptageniidae	↑ 9***	↓ 1	1.6	1.8
Goeridae	↑ 255***	↑ 387***	1.4	1.5
Baetidae	↑11***	↑ 12***	1.1	1.0
Hydropsychidae	↓ 8***	↓ 2	1.1	1.0
Perlodidae	↑ 11	↓ 32	2.6	2.5
Lymnaeidae	↓ 40	↑ 34	1.6	2.4
Glossiphoniidae	↓ 36	↑ 17	1.1	2.3
Erpobdellidae	↑ 29	↓ 1	0.3	2.3

Note: ↑, increasing trend; ↓, decreasing trend; NA, absent.
$p < 0.001$; **$p < 0.01$; *$p < 0.05$.***

significantly in newly invaded streams (S_1) but changes were evident in streams invaded by crayfish for ~20 years (S_3).

The negative effects of signal crayfish on several invertebrate taxa (Nilsson et al., 2012; Nyström et al., 1996) have already been recognised. Crayfishes, including signal crayfish, can alter invertebrate community structures directly, primarily through predation, or indirectly via trophic cascades (Bondar et al., 2005; Jackson et al., 2014). However, similar to fish communities, our 2011 and 2018 macroinvertebrate data represent a limited temporal context.

Long-term (1990–2017) Tees data showed that despite similar taxonomic richness in macroinvertebrate communities between invaded and uninvaded sites before signal crayfish invasion, richness declined significantly after invasion. Researchers have reported differential effects of invasive crayfishes on slow-moving taxa and more mobile taxa (e.g. Mollusca, Hirudinea, and case-bearing caddis flies; Dorn, 2013; Keller & Ruman, 1998; Parkyn et al., 1997; Wilson et al., 2004), and our results also show community shifts to lower abundances of some slow-moving taxa. The negative impacts of crayfish on molluscs are probably the most often reported (e.g. Lodge et al., 1994; Mathers et al., 2016; Nyström et al., 2001; Weber & Lodge, 1990). In this study, the pulmonate snail family Ancylidae decreased significantly following signal crayfish invasion whereas an opposite trend was observed in uninvaded streams. The bivalve mollusc family Sphaeriidae decreased in invaded streams but increased in uninvaded streams. Abundance of both families was significantly higher in uninvaded sections compared to signal crayfish-invaded sections within the same streams. This may primarily be due to direct consumptive effects by the crayfish (Dorn, 2013; Wilson

TABLE 6 Status of habitat and parameters over time (study S_1, 2011 vs. 2018) at before–after/control–impact survey sites, obtained through linear mixed-effects modelling

	Stream categories					
	Uninvaded		Newly invaded		Pre-invaded	
Parameters	*F*	*p*	*F*	*p*	*F*	*p*
Water depth	1.14	0.327	2.39	0.262	0.69	0.433
Flow velocity	2.98	0.135	2.61	0.248	1.73	0.230
Bottom substrates						
Boulder	1.78	0.231	1	0.423	1	0.351
Cobble	4.50	0.078	0.68	0.498	2.15	0.186
Pebble	2.21	0.188	0.48	0.560	0.21	0.660
Gravel	0.94	0.370	0.08	0.802	0.06	0.810
Sand	5.26	0.062	0.40	0.594	5.25	0.056
Flow types						
Pool	1	0.360	0.08	0.802	1.75	0.228
Riffle	1.22	0.311	1	0.422	3.37	0.109
Glide	2.16	0.192	0.01	0.936	2.03	0.197
Cascade	1	0.356	1	0.423	2.27	0.176
Others						
Substrate embeddedness	2.4	0.172	0.14	0.742	1.57	0.23
Canopy cover	0.3	0.604	1	0.423	0	1

et al., 2004) as the limited locomotion of gastropods makes them very susceptible to crayfish predation (Hanson et al., 1990; Rosewarne et al., 2013). However, molluscs such as these are slow-moving benthic invertebrates and a decrease in their abundance in response to crayfish invasion is in accordance with our hypothesis regarding greatest impacts on the most sensitive taxa.

The stonefly family Perlidae decreased in both stream categories but significantly in crayfish-invaded streams. A reduced number of stonefly (Plecoptera) has reported earlier from the crayfish-invaded parts of the river in Scotland (Crawford et al., 2006). However, several stonefly families are also predatory groups that could potentially compete with small crayfish and their abundance may be more in places with no crayfish (Ruokonen et al., 2014). Caddisflies exhibited increases or decreases in abundance depending on the families. Lepidostomatidae, Polycentropodidae, and Rhyacophilidae reduced significantly following signal crayfish invasion but the latter two taxa also decreased in uninvaded streams. The opposite trend occurred for Glossosomatidae and Goeridae. This may be due to variation in external protection; the latter groups are case-bearing caddisfly and they make hemispherical portable cases entirely made of sand grains and silk (Becker, 2001; Cox & Wagner, 1989; Nijboer, 2004) that offer more protection against predators, compared to Polycentropodidae and Rhyacophilidae, caseless caddisfly families.

The abundance of the amphipod family Gammaridae remained similar in crayfish-invaded and uninvaded streams. No significant difference in abundance of this family was recorded between invaded and uninvaded parts of the same streams. The abundance of this group is also reported to remain unchanged following signal crayfish invasion in lowland rivers of the U.K. (Mathers et al., 2016), but not always in upland streams (Crawford et al., 2006). The adoption of various avoidance strategies by this group (e.g. enhanced drift and locomotion, vertical migration and increased use of refuges) enables them to successfully evade inter- and intra-specific predation (Andersson et al., 1986; Haddaway et al., 2014; McGrath et al., 2007).

5 | CONCLUSIONS

This study supports our hypothesis that native fishes, especially benthic species, and potentially YoY salmonids, and less mobile macroinvertebrate taxa, are declining in response to non-native signal crayfish invasion in upland English streams. This is not due to habitat change, and water quality has remained good or, generally, improved and cannot be considered causal either. Impacts of non-native crayfish may not be evident immediately after colonisation, because of their slow invasion rate during establishment and more rapidly thereafter (Bubb et al., 2005; Guan & Wiles, 1996; Peay & Rogers, 1999). Nevertheless, a considerable reduction in abundance of the recipient communities may be evident, as has happened for the newly invaded streams in this study. This study also concludes that, in a signal crayfish-invaded stream, macroinvertebrate community will be impacted first, followed by the fish community, as we recorded significant changes in macroinvertebrates in streams invaded for less than 7 years (i.e. newly invaded streams in S_1) but no such change in fishes over the same timescale. In streams invaded by crayfish for over 8 years negative

TABLE 7 Long-term comparison (Study S_3, 1990–2018) of changes in water quality parameters over before and after signal crayfish invasion in invaded ($n = 4$; Alwent Beck, Deepdale Beck, River Balder, and River Lune) and uninvaded streams ($n = 2$; Clow Beck and River Greta), obtained through linear mixed-effects modelling (LMM)

Parameter	Group	Stream	LMM results	
			F	*p*
Dissolved oxygen	Invaded	Alwent	0.31	0.582
		Balder	0.17	0.687
		Deepdale	0.36	0.559
		Lune	0.63	0.433
	Uninvaded	Clow	1.72	0.205
		Greta	1.02	0.324
Biochemical oxygen demand	Invaded	Alwent	4.79	**0.031 ↓**
		Balder	3.23	0.086
		Deepdale	7.64	**0.008 ↓**
		Lune	7.13	**0.014 ↑**
	Uninvaded	Clow	13.47	**0.002 ↓**
		Greta	0.003	0.959
Hardness	Invaded	Alwent	1.96	0.199
		Balder	7.75	**0.012↓**
		Deepdale	2.28	0.140
		Lune	1.75	0.203
	Uninvaded	Clow	0.10	0.760
		Greta	2.38	0.128
Nitrogen	Invaded	Alwent	1.37	0.252
		Balder	4.46	**0.045 ↓**
		Deepdale	0.20	0.657
		Lune	4.60	**0.043 ↓**
	Uninvaded	Clow	5.86	**0.024 ↓**
		Greta	1.26	0.285
Ammonia	Invaded	Alwent	4.38	**0.038**
		Balder	5.51	**0.027 ↓**
		Deepdale	0.24	0.628
		Lune	0.50	0.489
	Uninvaded	Clow	1.87	0.184
		Greta	3.22	0.094
pH	Invaded	Alwent	6.66	**0.015 ↑**
		Balder	0.36	0.555
		Deepdale	2.55	0.129
		Lune	4.04	0.057
	Uninvaded	Clow	4.66	**0.039 ↑**
		Greta	0.87	0.361
Water temperature	Invaded	Alwent	0.47	0.493
		Balder	0.48	0.491
		Deepdale	0.74	0.393
		Lune	0.09	0.764
	Uninvaded	Clow	0.42	0.519
		Greta	0.002	0.960
Turbidity	Invaded	Balder	8.25	**0.005 ↓**
		Lune	14.40	**<0.001 ↑**
Zinc	Invaded	Alwent	1.70	0.206
		Balder	2.74	0.109
		Deepdale	0.06	0.808
		Lune	0.42	0.520
	Uninvaded	Clow	1.11	0.307
		Greta	1.25	0.269

Note: Bold values indicate outcomes significant at $p \leq 0.05$.
Linear model summaries are available in Table S9 and scattergraphs in Figures S2–S10. Where trends are significant the direction is indicated with an arrow.

effects on fishes, especially bullhead, were evident and the macroinvertebrate community was found to be severely impacted.

Population characteristics (density, size distribution, and sex ratios) of the invading signal population in the upper Tees show that it is still expanding rapidly, although some stream subpopulations are probably now close to carrying capacity. This may pose a major threat to the native fish populations, particularly to the benthic bullhead and may result in local extinction of the species, as recorded in two pre-invaded streams of this study. It is possible that recruitment of trout populations in these rivers may also be impacted as signal crayfish approach carrying capacity, but longer-term BACI studies are needed to study this more fully. The temporal pattern of fish and invertebrate impacts observed in our study supports our hypothesis that disruption of ecological conditions in signal crayfish-invaded streams will be greatest as crayfish reach carrying capacity.

Findings from this study may help in determining an appropriate strategy for managing crayfish invasions in relation to fisheries and conservation (Moorhouse et al., 2014) where natural, rather than stocked, salmonid fisheries are being encouraged (Peay et al., 2009). Our findings suggest that widespread and long-term ecological disruption is occurring in upland streams and that preventing further introductions and spread of non-native crayfish is crucial to limiting the extent of those impacts.

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DATA AVAILABILITY STATEMENT

Data from this research are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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